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Zahdi Date Vinegar: Production and Characterization

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ABSTRACT

The "Date Strategy Report" indicated that 14.3% of the total production in Iraq is wasted and 35.7% of the date crops are sold as animal feed to the dairies and sheep herders. To reduce these loses, a study program was initiated to investigate the suitability of low cost Zahdi date for natural vinegar production. The production of "Zahdi" vinegar was carried out by a two-step fermentation system; anaerobic conversion of sugars to ethanol using *Saccharomyces cerevisiae* and aerobic oxidation of ethanol to acetic acid at 30 °C using *Acetobacter*. The results indicated that 1 kg of low cost Zahdi date produced 4.30±0.35 L of 6.47±0.20% (w/v) ethanol in less than 96 h. The acetic fermentation of this wine for 12-14 days resulted in 4.05±0.27 L of natural vinegar containing up to 6.62±0.15% of acetic acid (w/v) with a fermentation efficiency of 90.50±1.70%. The concentrations of Na, K, Ca, Mg, Fe, Mn, Cu, Zn, Cd and Pb in Zahdi homemade vinegar and 21 commercially available vinegars were investigated as well. The results indicated that Zahdi dates vinegar could be considered as safe and as a good additional source of the essential nutrients.

Key words: Iraqi Zahdi dates, ethanol, vinegar, gas chromatography, atomic absorption spectroscopy, fermentation kinetic parameters

INTRODUCTION

The fruit of the date palm (*Phoenix dactylifera*) is an important commercial crop in Middle East countries. The date fruit consists of 70% carbohydrates, making it one of the most nourishing natural foods available to man. Compared to other fruits and foods; apricot 520, banana 970, orange 480, cooked rice 1800, wheat bread 2295 and meat (without fat) 2245 kcal kg^{-1}, respectively, dates give more than 3000 kcal kg^{-1} (Zaid and Wet, 2002). The energy requirements of adult men range from 2300-2900 kcal day^{-1} and 1900-2200 kcal day^{-1} for adult women. Hence, a portion of 100 g of dates supplies approximately 12-15% of the total energy requirement per day per adult (Al-Farsi and Lee, 2008). Dates are also a good source of vitamins and may be considered as the richest source of macrominerals such as Ca, P, Mg and K and microminerals such as Fe, Zn, Cu, Mn and Se compared to any other commonly consumed fruits (Vayalil, 2012; Al-Gboori and Krepl, 2010; Al-Shahib and Marshall, 2003; Mrabet *et al*., 2008).

Iraq has historically been one of the major date-producing countries in the world. In the 1980s and mid-1990s, Iraq was consistently among the top five date-producing countries in the world and often ranked No. 1 in terms of production by volume. Into the 1990s, Iraq had 22 million date palms planted over 120,000 ha, producing around 900,000 tons (Zaid and Wet, 2002; USAID, 2008). Date-production in Iraq is dominated by the Zahdi variety (dry) that represents 72% of total date production, followed distantly by Khastawi (fresh), Sayer (dry) and Khadrawy (semi-dry).

Zahdi date is medium in size, cylindrical, light golden-brown, very sugary and distinguished by its large seed in proportion to the fruit itself. This date is known for its high invert sugar level and is widely used to make date sugar products.

The wars, sanctions and general turmoil within Iraq over the past 20 years has led to the failure of the date production to a historical low of only 350,000 tons in 2007 and industry during a period when other countries were investing heavily in both date production and modern processing facilities (USAID, 2008). The "Date Strategy Report" indicated that 14.3% of the total production in Iraq is wasted and 35.7% of the date crop are sold as animal feed to the dairies and sheep herders. Low-quality dates are sold in the domestic market for approximately USD 100 per ton and exported at prices of USD 75-150 per ton (USAID, 2008). This is an intolerably great loss for Iraq, which is poor in food resources after two destructive wars and a decade of debilitating sanctions. Fermentation of low cost dates juice to natural vinegar, however, can be a feasible solution to avoid this loss.

Natural vinegar is a superior food additive as it contains nutrients such as carbohydrates, amino acids and peptides, vitamins and minerals and non-nutrient substances, such as carotenoids, phenolic compounds and some other pigments (Guerrero *et al*., 2007). From the early days of agriculture until today, mankind has always used vinegar for the same purposes as a; condiment, pickling or preserving agent, disinfectant, cleaning agent and beverage, with virtually no exceptions for all the cultures in the world.

These benefits stimulated intensive studies on the fermentation of guava, banana, onion, kiwi, orange, cajá, cashew (Silva *et al*., 2007), strawberry and persimmon surpluses (Ubeda *et al*., 2010), Mango (Ameyapoh *et al*., 2010), pineapple (Sossou *et al*., 2009) and sugarcane (Kocher *et al*., 2006). In contrast, there is a general lack of information concerning the suitability of dates for vinegar production although, traditional vinegar produced from fermented dates is very popular in Iraq and many other Mediterranean countries (Barreveld, 1993; Benamara *et al*., 2008).

Based on these considerations, the objective of this study was to investigate the suitability of low cost Iraqi Zahdi dates for vinegar production to offer dates producers another alternative to diversify their production. Zahdi vinegar was produced by a two stage fermentation process: (1) Conversion of fermentable sugars to ethanol by a commercial baker's yeast and (2) Oxidation of ethanol by old vinegar (Tesfaye *et al*., 2002; Solieri and Giudici, 2009). The levels of ethanol, acetic acid and 10 major and minor elements in Zahdi homemade and 21 commercially available vinegars were monitored by gas chromatography, titrimetry and atomic absorption spectroscopy.

MATERIALS AND METHODS

Preparation of Zahdi juice: Low cost dates of the most abundant cultivars in Iraq, Zahdi were obtained from the local markets at the time of harvesting in the year 2013 and were stored at -18°C until used for the experiments. Each date weighed about 5-7 g per fruit with seeds to date fruit ratio of 13±2% w/w at the tamar stage (full ripeness). The date fruits were cleaned, pitted, cut into small pieces and extracted trice with distilled water (2:1) at 70°C. The extracted juice was filtered and then concentrated to the required concentrations using rotary evaporator at 70°C.

Fermentation processes: The clarified Zehdi juice was placed in 3 L plastic flasks with 2.0 L juice per flask and inoculated at 30°C through the addition of a Chinese bakery yeast (*Sacharomyces cerevisiea*), that allows rapid and reliable fermentations (Converti *et al*., 2003; Aranda *et al*., 2011; Kumoro *et al*., 2012). The fermented juice was centrifuged at 4000 g for 20 min and then acetified by old vinegar in the proportion of 1-10 parts of the date wine in line with the surface culture method usually known as Orleans technique (Hutkins, 2006). The containers used have a wide surface area, so that the bacteria have enough oxygen to do their work. As a general criterion, alcoholic fermentation was considered to have finished when the sugar had been consumed (<0.5% w/v) and ethanol concentration had fallen below 0.50% (w/v).

Commercial vinegars: Twenty one vinegar samples of different sources, representing the common types of vinegars readily available to Iraqi consumers, were obtained from retail stores. The majority of the vinegars were in glass bottles however, several were in plastic bottles. Each sample was filtered and centrifuged at 4000 g for 20 min before the analysis.

Physiochemical analysis: At appropriate time intervals, samples from the homemade fermenter were centrifuged at 4000 rpm for 20 min. The supernatant was used to determine the total acidity, pH, conductivity, total soluble solids, total soluble sugars and ethanol. Total acidity was evaluated by titration with standardized solution of 0.1 M sodium hydroxide using phenolphthalein as indicator and the results were expressed as acetic acid content. The acidity of vinegar is mainly due to the presence of acetic acid and smaller amounts of other acids come from raw materials or are generated by the fermentation (Aguiar *et al*., 2005). The pH and conductivity of the samples were measured using a pH-meter (Model 240, WTW) and a conductimeter (Model 300 WTW), respectively. The percentage (w/v) of soluble solids (°Brix) was determined with Abbe refractometer (Novex, 98.490, Holland), whereas total soluble sugars were analyzed by the phenol-sulphuric acid method (Dubois *et al*., 1956).

Ethanol determinations were carried out by a Shimadzu-2010 gas chromatograph equipped with flame ionization detector and Zebron column (ZB-FFAP, length 30 m, I.D. 0.25 mm, film thickness 0.25 μm). Normal butanol was used as an internal standard. The operational temperatures for the injector, detector and column were 120, 120 and 90°C, respectively. Ultra-pure nitrogen carrier gas with a flow velocity of 2.4 mL min^{-1} and sample volumes of 1 μL injected into split mod (1:20) were utilized. Ethanol was also estimated by potassium dichromate oxidation method using spectrophotometry (Wang *et al*., 2003).

The ethanol and acetic acid productivity (g L^{-1} h^{-1}) was calculated as the amount of ethanol or acetic acid production over the total fermentation time. The ethanol and acetic acid yield (fermentation efficiency) were defined as the ratio of the total alcohol or acetic acid produced to the theoretical ethanol or acetic acid production, which was stoichiometrically calculated from the consumed sugars or ethanol. ANOVA analysis was performed on the data using SPSS version 17.0 for windows. Significance was accepted at $p<0.05$.

Elemental analysis: An aliquot of approximately 50 mL of homemade or commercial vinegars were accurately weighed into a high-form porcelain crucible and carefully evaporated to dryness. The dry extract obtained was introduced into a muffle furnace and the temperature slowly increased to 550°C. The white ash obtained (after 12 h) was dissolved in 10 mL of 0.25% nitric acid and the resulting solutions were analyzed using Shimadzu 6300 atomic absorption spectrometer equipped with Graphite Furnace Atomizer GFA-EX7i. The contents of calcium, magnesium, iron, manganese, copper and zinc in the samples were analyzed by FAAS and the sodium and potassium content by FAES, while the concentrations of cadmium and lead were measured by GFAAS. Standard aqueous solutions of different elements obtained from Fisher Scientific Company, USA were used to calibrate the AAS machine.

All the experiments were carried out three times (triplicate) and the mean value of the three experiments was presented. The reliability of the method used for the estimation of Cd, Pb, Zn, Cu, Mn and Fe concentration in vinegar samples by AAS technique has been checked by stripping voltammetric technique using a Metrohm 797 VA Trace Analyzer. The accuracy was also checked with recovery assays by adding known amounts of analyte to five different vinegars prior to the digestion step and processing those samples in the same way as other experimental samples. Mean recoveries of spikes were in the range from 91.3-109.6% for the investigated elements.

Glassware was cleaned by soaking in 10% v/v HNO_3 for 24 h and rinsing at least three times with ultra pure water. Then, the material was dried and stored in a class 100 laminar flow hood. All chemicals and solvents used were of analytical grades and purchased directly from Merck (Darmstadt, Germany).

RESULTS AND DISCUSSION

Alcoholic fermentation: Approximately 4.30±0.35 L of juice containing 15.35±0.32% (w/v) total soluble sugars were obtained from 1 kg of low cost Zahdi dates. The initial pH of the juice was 5.53 and the titratable acidity as acetic acid ranged from 0.31-0.46% (w/v). It was not necessary to make any pH correction of the juice because no significant difference was observed among the initial pH tested (4.5, 5.0 and 5.5) regarding ethanol production.

Since non-optimal conditions can stress the yeast and reduce the productivity (Bai *et al.*, 2008), the effect of both initial sugar and yeast concentrations were investigated. The effect of sugar concentration was followed by varying substrate concentration from 8-45%. The samples were filtered and inoculated with 0.5% (w/v) of baker's yeast at 30°C (with no nutrient addition). The fermented juices showed vigorous fermentation capacity within 2 h and reached maximum ethanol concentrations of 3.29-10.30% w/v in 48-96 h. The highest efficiency range (81.66-85.01%) was obtained at a sugar concentration range of 8-20% (w/v) (Fig. 1). An adverse effect was observed beyond 20% (w/v) due to osmotic stress (Ndip *et al.*, 2001). This finding is in good agreement with several workers who found a reduction of fermentation rate as a result of drastic increase in initial sugar concentration using different substrates (Bai *et al.*, 2008; Arroyo-Lopez *et al.*, 2009; Attri, 2009; Kumoro *et al.*, 2012). Furthermore, the results indicated that higher sugar concentrations slightly prolong Zahdi juice fermentation. Similar trend was previously reported by Borzani *et al.* (1993) who found an exponential relationship between the time necessary to complete the fermentation of sugarcane molasses and the initial concentrations of sugar and yeast cells.

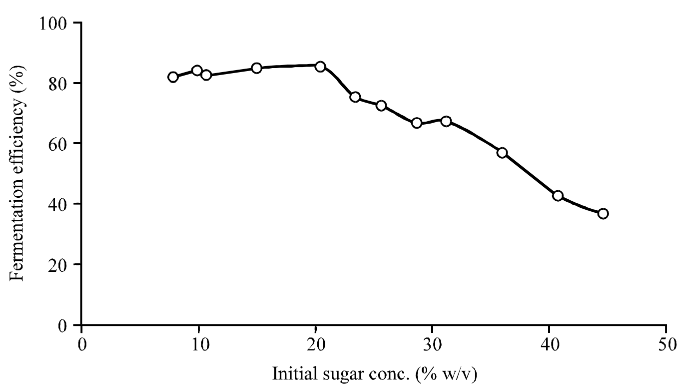

Fig. 1: Effect of increasing sugar concentration on ethanol production efficiency at 30°C

Table 1: Ethanol production from Zahdi juice containing 15% (w/v) sugar as a function of baker's yeast concentration and time at 30°C

	Ethanol concentration (%) (w/v)			
	Time (h)			
Bakers yeast concentration (g L^{-1})	24	48	72	96
5.0	4.26	5.56	5.82	6.47
10.0	5.26	5.61	6.13	6.75
15.0	5.98	6.18	6.50	6.78
20.0	6.19	6.48	6.36	6.44
30.0	5.38	6.22	6.16	6.25

The fermentation efficiency range of Zahdi juice in the present study is close to those reported by Nofemele *et al.* (2012) for sugarcane molasses (85.12%) and Ilha *et al.* (2000) for honey bee (81.34%) whereas, it is higher than those reported by Silva *et al.* (2007) for cashew and kiwi wine (57.7%), Arumugam and Manikandan (2011) for banana and mango fruit wastes pulp (52.0-70.3%) and Siqueira *et al.* (2008) for soybean molasses (45.4%).

The concentration effects of *Saccharomyces cerevisiae* on growth and ethanologenic fermentation performance of date juice were studied at 30°C and presented in Table 1. The results reveal that a yeast concentration of 5 g L^{-1} was adequate to produce 6.47% (w/v) ethanol from Zahdi juice containing 15% (w/v) soluble sugar in 96 h. The results of this study are consistent with previous observations by Kumoro *et al.* (2012).

Increasing yeast concentration from 5-20 g L^{-1} gave faster production rate (2.579 g L^{-1} h^{-1}) within the first 24 h. Further increase in the yeast concentration to 30 g L^{-1} resulted in minor inhibition of the ethanol yield. Such behavior is usually attributed to the fact that when the yeast cells concentration is too high, the competition amongst yeast cells to consume nutrients becomes very high (Ndip *et al.*, 2001).

The increasing demand for ethanol for various industrial purposes such as alternative source of energy, industrial solvents, cleansing agents and preservatives has necessitated increased production of this alcohol. The worldwide production of bioethanol reached around 51 billion liters in 2006, of which 18 billion liters were produced in USA from maize (Siqueira *et al.*, 2008). A worldwide interest in the utilization of bio-ethanol as energy source has stimulated studies on the cost and efficiency of industrial processes for ethanol production. In this study, 1 kg of low cost Zahdi date produced about 4.30 L of 6.48% (w/v) ethanol in less than 48 h using 20 g L^{-1} of baker's yeast and approximately the same yield in 96 h using 5 g L^{-1}. Scale-up studies need to be carried out for an actual assessment of the cost of production of dates wine. However, because the production cost of these alcoholic extracts from low cost date are inexpensive, it is very much attractive for using in natural vinegar production.

Acetic fermentation: Acetic fermentation is the next step in vinegar production, where alcohol molecules are oxidized into acetic acid molecules by the action of *Acetobacter*, giving it the characteristic vinegar taste (Fig. 2). Zahdi wine (4.30 L), obtained from the fermentation of 1 kg Zahdi juice was diluted to 5.58% (w/v) with old vinegar and used as starting material for vinegar production at 30°C. In 12-14 days of acetic fermentation, about 4.05±0.27 L of natural vinegar was produced from five replicates of the experiment, containing 6.62±0.15% (w/v) of acetic acid and less than 0.50% (w/v) of alcohol. According to the stoichiometric reaction of ethanol transformation into

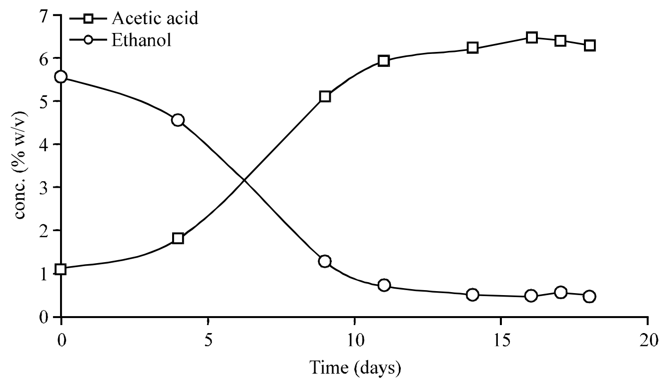

Fig. 2: Profiles of acetic acid production and ethanol consumption during 18 days of acetic fermentation by baker's yeast

acetic acid, where 1 g ethanol yields 1.304 g acetic acid, 5.58% (w/v) of ethanol consumed at the medium should yield 7.28% (w/v) acetic acid. Practically, the concentration reached in the present study was 6.62±0.15% (w/v) acetic acid at a fermentation efficiency of about 90.50±1.70%. This high efficiency is close to those reported by other workers using different substrates such as bees and mango juice (Ameyapoh *et al*., 2010; Ilha *et al*., 2000). The residual alcohol is partially transformed into vinegar and partially converted into esters during storage and giving the vinegar the characteristic flavor of the raw material utilized in the acetification process.

In the traditional procedure used in Iraq and many other Mediterranean countries, the production of vinegar depends on the addition of about 5% (w/v) non-pasteurized old vinegar directly to the date juice at the start of fermentation. The organisms concerned usually grow at the top of the substrate and form a jelly like mass, composed of both *Acetobacter* and yeast working together. In this (spontaneous) fermentation process, significant differences were emerged, firstly 1 kg of low cost date produced 3.25 L of 4.02% (w/v) acetic acid in about 33 days and secondly, a significant amount of sugar (5.34%) remained in date vinegar at the end of its production. Similar observations were reported by other workers using different substrates (Sossou *et al*., 2009). This low yield is expected as the use of undefined cultures, in one step fermentation system, does not ensure total control of the acetification or the quality of the product (Hidalgo *et al*., 2010; Krusong and Vichitraka, 2010). Weak lipophilic acids and low pH have a synergic effect and expected to reduce intracellular pH below the normal physiological values, inhibiting the yeast growth. Moreover, acetic acid seems to directly affect transport or enzymatic activities, such as enolase, a key enzyme of glycolysis (Valli *et al*., 2005; Graves *et al*., 2007).

The product from the Orleans process is high quality vinegar since, the slow production process promotes the development of flavor and aroma (Raspor and Goranovic, 2008). Additionally, production by the Orleans method can be performed as a semi-continuous process because once the vinegar has developed to a desired quality, about half to three-fourth of the volume of the finished vinegar can be removed from the bottom while the same volume of non-acetified alcoholic substrate is simultaneously added from the top (Mazza and Murooka, 2009). The bacteria in the vinegar, that is left in the fermenter, can be used as inoculate for the next production volume.

Commercial vinegars analysis: Table 2 shows the physiochemical parameters of 21 commercial vinegars currently consumed in Iraq. The pH of the samples presented values around 2.80 which

Table 2: pH, electrical conductivity, total titratable acidity, ethanol content and total soluble sugar for homemade vinegar and 21 commercial vinegars currently consumed in Iraq*

					(% w/v)		
Vinegar label	Type	Source	pH	Electrical conductivity (μ cm^{-1})	Acidity	Ethanol	Total sugar
Homemade	Date	Iraq	3.07	7480	7.24	0.22	0.66
Commercial							
Albadawi	Date	Iraq	2.96	3270	2.85	<0.01	0.29
Ahla Baghdad	Date	Iraq	2.84	1815	3.18	0.04	0.03
Al Arabi	Date	Iraq	3.03	2610	3.18	0.25	0.19
Ibin Hayan	Date	Iraq	3.02	3350	5.28	1.44	1.17
Hello	Artificial	Iraq	2.65	1522	4.86	0.03	nd
Albadawi	Artificial	Iraq	2.57	1607	4.56	<0.01	2.47
Sham gardens	Date	Syria	2.99	3910	5.26	2.53	0.58
Durra	Date	Syria	3.23	5430	4.22	1.07	1.73
Mayas	Ginger	Syria	3.26	3860	5.04	2.88	0.75
Sham gardens	Grape	Syria	2.95	2980	4.63	0.50	0.46
Al Walaem	Garlic	Syria	3.12	4260	4.98	<0.01	0.55
Alwadi alakhdar	Grape	Lebanon	2.86	2840	5.64	0.18	0.21
Hamra (White)	Grape	Lebanon	2.49	1316	6.18	<0.01	nd
Hamra (Red)	Grape	Lebanon	2.55	1315	5.76	<0.01	nd
Teeba Al Jabal	Artificial	Lebanon	2.52	1331	5.34	<0.01	nd
Zer	Apple	Turkey	2.71	1729	5.17	0.09	1.48
Kemal Kukrer	Grape	Turkey	2.99	3450	5.11	<0.01	0.42
American garden	Vegetable	U.S.A	2.40	1573	6.12	0.16	2.47
American garden	Grape	U.S.A	2.53	1566	5.40	0.18	0.22
Baider	Sugarcane	K.S.A.	2.43	1571	6.36	<0.01	2.40
Hello	Artificial	Jordan	2.67	1512	4.08	<0.01	nd

*All averages derived from three readings with a maximum standard deviation of 5%, (%) (w/v): g/100 mL, nd: Not detected

is similar to those reported by other workers (Aguiar *et al*., 2005). Excluding 3 brands from Iraq (Albaldawi, Ahla Baghdad and Al Arabi) all analyzed vinegars presented minimum acidity of 4.00% value that is demanded by the North American legislations and confirmed in the labels of 90% of the samples (FDA, 2007).

On the other hand, 76.2% of the commercial vinegars had alcohol contents between trace and 0.50% (w/v), whereas 23.8% contained significantly higher ethanol concentration of 0.50-2.88% (w/v). The highest concentration was detected in Mayas (2.87% w/v) and Sham gardens (2.53% w/v) from Syria. These values are much higher than the maximum ethanol content (0.5 and 1% v/v) set by the Codex Alimentarius commission for wine vinegar and other vinegars (CAC, 2000).

The electrical conductivity measurement of the samples (Table 2) indicated that the home made and all the commercial vinegars studied are characterized by relatively high electrical conductivity values (1315-7480 μS cm^{-1}). There is no WHO or EEC guideline value derived for the permissible or maximum allowable level of electrical conductivity or TDS for vinegar.

Trace elements analysis: The presence of mineral constituents in wines and vinegars is related to factors such as the natural composition of the substrates, contact with production and storage equipment and contamination of the environment. The high concentration of certain elements can induce undesirable processes, such as precipitation, change in color and turbidity

Table 3: Average* metal contents for homemade Zahdi vinegars (mg L^{-1}) and their typical substrates: Pulp, baker's yeast (mg kg^{-1}) and juice**, wine (mg L^{-1})

	Metal contents									
Varieties	Na	K	Ca	Mg	Fe	Mn	Cu	Zn	Cd	Pb
Pulp	436	7744	1140	236	8.40	3.41	2.503	2.62	0.001	0.096
Juice	162	2196	312	64	3.26	0.92	0.541	0.88	0.003	0.071
Wine	156	1878	273	56	2.08	0.66	0.223	1.03	0.089	0.111
Vinegar	148	1958	293	50	1.15	0.49	0.078	1.29	0.069	0.127
Yeast	1636	16909	2147	582	91.10	10.20	1.611	130.00	0.092	2.353

*All averages derived from three readings with a maximum standard deviation of 5%, **15% w/v total soluble sugars

(Guerrero *et al*., 1996). The presence of elements is also important due to their potential toxicity and they can be used to characterize the geographical origin of the fruit used for fermentation (Guerrero *et al*., 1997).

Generally, data on the mineral content in wines (excluding date's wine) have been extensively studied and reported due to their implications in organoleptic, hygienic and dietetic characteristics. Nevertheless, only a few studies are found in literature concerning the mineral concentration of traditional vinegars (Guerrero *et al*., 1996; Laurie *et al*., 2010).

Table 3 shows the average content of Na, K, Ca, Mg, Fe, Mn, Cu, Zn, Cd and Pb elements determined in Zahdi date pulps. The results clearly indicates that dates pulp could be considered as a good source of iron, potassium, copper and manganese and a fair source of calcium and magnesium and suggests the potential use of dates in many mineral deficiency disease (Vayalil, 2012). The results obtained in the current study are comparable with previously reported values for different types of dates (Vayalil, 2012; Al-Farsi and Lee, 2008; Al-Gboori and Krepl, 2010; Al-Shahib and Marshall, 2003; Mrabet *et al*., 2008). Table 3 also lists the concentration of the elements at different stages of the homemade vinegar making process (i.e., date juice, wine and vinegar). The data indicate that the average metal contents are almost the same when using glass or plastic ware. This result is anticipated since the mineral content is not affected by fermentation unless some salts are added to the product during fermentation or when fermentation is carried out in metal containers and some minerals are dissolved by the fermented products (Guerrero *et al*., 1997).

The mineral compositions of the Baker yeast used throughout the fermentation process are also presented in Table 3. Based on these levels, the addition of 5 g of Baker yeast to 1 L of fermented juice would not promote significantly the average mineral content of the homemade vinegar. Consequently, the date vinegar prepared in the present study may be considered as a suitable reference for the studied imported vinegars and that individual metallic contents seem to be suitable descriptors for wine vinegar characterization.

The concentrations of Na, K, Ca, Mg, Fe, Mn, Cu, Zn, Pb and Cd elements in 21 commercial vinegars are presented in Table 4. The average values for each metal were Na 106, K 323, Ca 227, Mg 44, Fe 4.64, Mn 0.68, Cu 0.144, Zn 0.39, Cd 0.058 and Pb 0.336 mg L^{-1}, respectively. A high variability in the concentrations of the elements can be noted due to the vinegar type and manufacturing process and also to the strong influence of the soil type and agricultural practices on the content of these elements in the fermented substrates (Navarro-Alarcon *et al*., 2007).

Of the elements present in the highest concentrations, potassium was the highest in all natural vinegar analyzed. The high concentrations of potassium reflect the high levels of potassium in the raw materials used in the production (Al-Gboori and Krepl, 2010). The results obtained in the

Table 4: Major and minor element composition* (mg L^{-1}) of different commercial vinegar samples currently consumed in Iraq

Name of vinegar	Na	K	Ca	Mg	Fe	Mn	Cu	Zn	Cd	Pb
Albadawi (Date)	140	430	299	74	12.05	0.36	0.184	0.51	0.055	0.592
Ahla Baghdad (Date)	100	120	66	32	9.40	0.14	0.114	0.28	0.037	0.029
Al Arabi (Date)	80	360	414	60	11.39	0.41	0.018	0.46	0.063	0.335
Ibin Hayan (Date)	110	690	816	69	14.68	0.41	0.141	0.49	0.142	0.041
Hello (Jordan) (Artificial)	330	3	nd	1	0.39	0.22	0.055	nd	0.124	0.064
Albadawi (Artificial)	70	36	146	24	0.21	0.20	0.050	0.04	0.051	0.351
Sham gardens (Date)	100	740	181	138	5.26	0.40	0.168	2.40	0.140	1.154
Durra (Date)	80	930	489	135	20.92	0.91	0.194	1.77	0.043	1.049
Mayas (Ginger)	164	930	204	47	12.79	2.10	0.183	0.26	0.051	0.147
Sham gardens (Grape)	60	580	789	67	1.79	0.93	0.130	0.25	0.061	0.112
Al Walaem (Garlic)	350	490	637	110	0.51	5.42	0.237	0.54	0.051	0.156
Alwadi alakhdar (Grape)	26	550	122	24	0.62	0.44	0.429	0.52	0.065	0.109
Hamra (white) (Grape)	20	1	27	3	0.21	0.33	0.290	0.11	0.019	0.103
Hamra (red) (Grape)	19	1	30	4	0.12	nd	0.189	0.06	0.019	0.180
Teeba Al Jabal (Artificial)	75	1	nd	nd	0.87	0.13	0.077	0.05	0.010	0.309
Zer (Apple)	70	180	140	34	1.75	0.27	0.104	0.07	0.031	0.054
Kemal Kukrer (Grape)	160	570	260	87	3.00	0.49	0.101	0.35	0.116	0.029
American garden (Vegatable)	29	23	53	1	0.21	0.30	0.104	0.03	0.055	0.885
American garden (Grape)	26	150	64	5	0.58	0.93	0.130	0.02	0.101	0.449
Baider (Sugarcane)	155	12	nd	nd	0.22	0.26	0.093	nd	0.029	0.113
Hello (Iraq) (Artificial)	70	1	38	17	0.53	0.24	0.066	nd	0.037	0.334

*All averages derived from three readings with a maximum standard deviation of 5%

present study are close to those reported in the literature for date vinegars but slightly lower than for wine and apple cider vinegar samples from Spain and Germany (Akpinar-Bayizit *et al*., 2010).

The values for sodium in the commercial vinegars varied over a wide range from 19-350 mg L^{-1} with an average value of 106.4 mg L^{-1}. These values are close to the data reported for different types of wine vinegars (Guerrero *et al*., 1996). Overall, the concentrations of sodium in all the samples analyzed are generally low and should not be considered as a health risk. Very high sodium levels were found in some artisanal homemade vinegars used by pickle sellers in Iraq, which needs urgent health supervision.

The interest of enologists in the sodium content of wine and vinegar started when it was reported that people suffering from hypertension should be on low sodium diets. Date fruit and vinegar, being exceptionally rich in potassium and extremely low in sodium, are a desirable foods for hypertensive persons who are advised to consume low sodium diets.

Calcium and magnesium, jointly with sodium and potassium, are the elements most absorbed by the date palm. While, the presence of Mg in wines and vinegars is mainly dependent on the natural content of their substrates, certain oenological treatments, such as the addition of calcium sulphate, clearly increases the level of calcium in wines and subsequently vinegars. The average calcium and magnesium contents of the commercial vinegars (227.3 and 44.3 mg L^{-1}, respectively) were lower than the values determined for the homemade Zahdi vinegars (Table 3) but within the range described in the literature (Navarro-Alarcon *et al*., 2007; Akpinar-Bayizit *et al*., 2010).

When a comparison was made between the mineral content of natural vinegars and distilled (artificial) vinegars, two significant differences were observed (Table 4). Firstly, a significant correlation was observed between potassium and magnesium ($r = 0.75$) and that the total potassium plus magnesium contents for the home made and natural commercial vinegars are much higher than those for artificial vinegars. Out of twenty one commercial vinegars, eighteen samples can be separated as labeled into two groups: One composed of eleven samples {Albadawi (natural), Al Arabi, Sham gardens (date), Durra, Ibin Hayan, Mayas, Sham gardens (grape), Al Walaem, Alwadi alakhdar, Zer and Kemal Kukrer} containing high K+Mg values between 214 and 1065 mg L^{-1}; other composed of seven brands (Baider, American garden (vegetables), Albadawi (artificial), Hello (from Jordan), Hello (from Iraq), Hamra (white), Hamra (red) and Teeba Al Jabal) with lower K+Mg concentrations of 1 and 60 mg L^{-1}. These directives can be adopted to discriminate between artificial and natural vinegars and could be used as an indicative of the raw material authenticity. Similar conclusion was previously reported by Rizzon and Miele (1998).

In recent years, there has been an intense and systematic effort made to apply novel techniques for accurate determination of wine and consequently vinegar authenticity and detection of possible adulteration. Certain elements in vinegars and wines such as Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn are being investigated, together with other physicochemical parameters in order to discriminate between wine and vinegar regions and types (Tesfaye *et al*., 2002; Du *et al*., 2012; Laurie *et al*., 2010). Calcium concentrations, however, should not be taken into account when determining the chemical composition of vinegars as Ca may be added during the enological process (Guerrero *et al*., 1996; Da Silva *et al*., 2007).

The average level of iron in Zahdi homemade vinegar was about 4.64 mg L^{-1} (Table 3). This level is within the range (1.95-10.50 mg L^{-1}) reported by Akpinar-Bayizit *et al*. (2010) for 35 commercial vinegar samples (including date vinegar) using inductively coupled plasma optical-emission spectrometry. On the other hand, the concentrations of iron in the commercial samples (Table 4), varied in range from 0.12 (Hamra red) to 20.92 mg L^{-1} (Durra). CAC (2000), state that the Fe content must not exceed 10 mg L^{-1}. In the present study, this level is exceeded in five commercial samples only. Knowing Fe level in vinegar is important because of its effect on undesirable process of oxidation which can give vinegar a dark and cloudy aspect (Galani-Nikolakaki *et al*., 2002). However, the tendency of vinegars to suffer browning is not widely demonstrated in the literature.

Mn is present in vinegar in very low concentrations, its content being characteristic of the production region and of the dithiocarbamic fungicide added. In the present study, the concentration of Mn ranged from trace to 5.42 mg L^{-1} (average 0.678 mg L^{-1}). These values are within the range obtained by others (Da Silva *et al*., 2007; Guerrero *et al*., 1996; Akpinar-Bayizit *et al*., 2010; Guerrero *et al*., 1997). Remarkably, Mayas (2.10 mg L^{-1}) and Al Walaem (5.42 mg L^{-1}) from Syria show relatively high values of Mn contents, probably due to the addition of growth activators for the bacteria used in the alcoholic fermentation (Rizzon and Miele, 1998; Tesfaye *et al*., 2002). Legal limitation for Mn concentration is not defined but it is important to know the concentration due to its great influence on wine oxidation and development of acetaldehyde during oxidation (Cacho *et al*., 1995).

Copper and zinc levels determined in the present study (0.018-0.429 and 0.001-2.399 mg L^{-1}, respectively) are within normal ranges according to previous data for vinegars from other word regions (Navarro-Alarcon *et al*., 2007; Silva *et al*., 2007; Akpinar-Bayizit *et al*., 2010). According to the CAC (2000), the total zinc+copper concentrations must not exceed 10 mg L^{-1}. All the vinegar samples in this study had copper+zinc contents below this limit. The presence of copper in vinegar may be due to corrosion of the tanks and metallic pipes by the acetic acid and from the sulfate treatment to which substrates are submitted. In 40 samples of wine vinegars derived from both slow and quick elaboration methods, Guerrero *et al.* (1997) determined the average contents of copper+zinc (1.7-6.4 mg L^{-1}) for slow elaboration. Their values were significantly higher than the values recorded in the present study, indicating that the metal content is closely related to the elaboration process and could be a suitable tool for vinegar characterization.

The Cu contents in all analyzed Zahdi date wines were lower than in date juices after clarification (Table 3). The decrease of copper concentration after the fermentation could be attributed to the yeast *Saccharomyces cerevisiae* which is well known to be an effective bioaccumulator of metals ions including copper (II) (Kristl *et al.*, 2003).

In contrast to Na, K, Ca, Mg, Fe, Mn, Cu and Zn, the concentration of Cd and Pb in wine and subsequently in vinegars is almost exclusively influenced by artificial sources. The lead and cadmium levels can be of anthropogenic origin due to residues of agrochemical products used as insecticides and fungicides and can also be due to environmental contamination due to contact with the apparatus used in their production and packaging process.

Both metals were present in all analyzed vinegar samples (Table 3 and 4), though in minor concentrations. The average Cd concentration in homemade and commercial vinegars (0.069 and 0.058 mg L^{-1}, respectively) was slightly lower than those reported (0.014-0.035 mg L^{-1}) by Acosta *et al.* (1993) and far lower than the upper level (1.00 mg L^{-1}) established by the CAC (2000). The average Pb concentrations in homemade and commercial vinegars were 0.127 and 0.336 mg L^{-1}, respectively. These values are close to the range (0.015 to 0.307 mg L^{-1}) reported by Ndung'u *et al.* (2004) for 59 types of vinegars, lower than the level (0.44-0.67 mg L^{-1}) given by Acosta *et al.* (1993) and far lower than (1.00 mg L^{-1}) established by the CAC (2000). Concentration above the Codex level was only found in two commercial date vinegars (Table 4); Sham gardens and Durra, probably due to their contact with contaminant lead surfaces during production and storage (Ndung'u *et al.*, 2004).

Based on an average daily consumption of 30 mL of homemade vinegar and the concentration of Cd and Pb (Table 3), the average daily intake of Cd and Pb from homemade vinegar are estimated to be 2.07 and 3.81 μg day^{-1}, respectively. The FAO/WHO Joint Expert Committee on Food Additives recommended a provisional maximum tolerable weekly intake (PTWI) of Cd and Pb from all sources (food, air and water) of 7 and 25 μg kg^{-1} body mass respectively (WHO, 1993). These values correspond to a provisional daily intake of 60 and 214 μg (assuming an average consumer weight of 60 kg). According to these levels, the daily intake of Cd and Pb from Zahdi vinegar alone is far below the FAO/WHO Provisional Tolerable Daily Intakes. It, therefore, appears that the consumers are not at any imminent health risk due to heavy metals from natural Zahdi vinegar examined in this study.

Cost economics: Vinegar is a very good preservative as well as condiment. It is used in many food preparations and snacks. It is used in large quantities in restaurants, clubs and canteens and by the caterers. Their purchased products are priced in the range of $1.00-3.00 per liter inside Iraq.

Approximately, 4 L of date vinegar with 6.62% (w/v) acetic acid and less than 0.50% (w/v) ethanol were obtained from 1 kg of low cost Zahdi date. Regulations in the United States require vinegar to contain at least 4% acetic acid resulting from acetic acid fermentation of ethanol containing substrates. Based on this acidic degree, approximately 6.6 L of natural vinegar can be obtained from only 1 kg of low quality Zahdi date costing less than $ 0.50 in the local market. For this reason, natural homemade vinegar would offer a very competitive price to all imported brands. However, scale-up studies need to be carried out for an actual assessment of the cost of production of Zahdi vinegar.

CONCLUSION

From this study, it can be concluded that:

- A two-step fermentation system was carried out as; anaerobic conversion of sugars to ethanol using *Saccharomyces cerevisiae* and aerobic oxidation of ethanol to acetic acid at 30°C using *Acetobacter* was much more efficient than the spontaneous and simultaneous fermentation in single cycle
- One kilogram of second quality Zahdi date yielded about 4.30 L of clear yellow-red wine, containing 6.47% w/v ethanol. The efficiency of the alcoholic fermentation was 80.00-85.00%. The acetic fermentation of this wine resulted in 4 L of natural vinegar containing 6.62% w/v of acetic acid and less than 0.5% w/v of alcohol representing an acetic fermentation efficiency of about 91% which can be considered of economical feasibility
- Zahdi dates vinegar could be considered as safe and a good additional source of the essential minerals investigated such as iron, manganese, magnesium and potassium
- The total K+Mg contents for the homemade and natural commercial vinegars are much higher than those for artificial vinegars. These directives may be used to discriminate between artificial and natural vinegars and could be used as an indicative of the raw material authenticity

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